active domains of FGF and hedgehog signaling during zebrafish organogenesis.

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Program/Abstract #77
Shh is required for the maintenance of postnatal mouse intervertebral disc

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Introduction: Degenerative disc disease is a major cause of lower back pain. However the molecular signals that control normal growth and differentiation of the disc are not well defined. We hypothesize that nucleus pulposus (NP) cells, which originate from the embryonic notochord, provide signals that control growth and differentiation of the postnatal disc. Methods: Postnatal day 4 mouse IVDs were cultured in serum-free DMEM Ham/F12 medium at 37 °C in 5% CO_2 for 2–5 days on type IV collagen-coated cell culture inserts. Cyclopamine (250 μM) was used as a Shh antagonist, and its specificity was tested by add-back experiments using 100 nM recombinant Shh (rShh). Cell proliferation was assayed using pulse-labelling with BrdU. 6 μm thick cryosections were immunostained (IHC) using antibodies specific to IVD differentiation markers, and Cy5 conjugated secondary antibodies. Imaging was carried out using confocal microscope. To test the role of Shh in vivo, triple transgenic mice [(tetO)7CMV-cre:R26 rtTA:Shhflx/flx] were injected with 2 μg of doxycycline on postnatal day 4 to conditionally delete Shh, and the lumbar spine was collected 5 days later. Results were compared to vehicle treated controls. Results: Both cyclopamine treatment in vitro and conditional Shh knockout in vivo caused rounding up and aggregation of the NP cells, and loss of orientation of the layers of the annulus fibrosus (AF). There was also a loss of expression of both NP and AF differentiation markers. BrdU incorporation studies showed loss of proliferation of NP cells. IHC for activated caspase-3 showed increased levels of apoptosis of NP cells rShh replacement and reversed the cyclopamine phenotype in vitro. Conclusions: These results show that Shh is essential for postnatal growth and differentiation of both the NP and AF. Significance: Identification of signals controlling IVD growth and differentiation offers the opportunity for the development of biological repair as an alternative to surgery.

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Program/Abstract #82
XTRIC-8, a protein required for proper neural crest formation

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The neural crest is a transient embryonic cell population that migrates extensively to various parts of the embryo, where it differentiates into diverse derivatives, including most of the cranio-facial skeleton and peripheral nervous system. Ric-8 has been characterized as a guanine nucleotide exchange factor (GEF) for heterotrimeric G proteins, and thus an activator for those proteins signaling pathways. In this work, we determined that in Xenopus tropicalis embryos, XtRic-8 is expressed in neural crest cells before and after the migration step takes place and later in neural crest derivatives as craniofacial arcs and otic vesicle. In order to study the function of XtRic-8 in neural crest formation, loss-of-function experiments in two-cell stage with antisense morpholino were carried out, resulting in an altered expression of neural crest markers. This result suggests that XtRic-8 is necessary for proper neural crest specification and migration. Loss-of-function experiment effects in neural crest formation could be due to the fact that XtRic-8 is also probably required for normal development during previous stages. On the other hand, transplantation assays, resulting in migration defects and cartilage staining assays, resulting in craniofacial defects, confirmed that XtRic-8 is required for proper neural crest formation.

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Program/Abstract #83
Tetraspanin18 restricts neural crest migration by modulating Cadherin6B mRNA and protein levels

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Unlike typical neuroepithelial cells in the developing central nervous system, neural crest cells undergo an epithelial to mesenchymal transition (EMT), detach from the neural tube, and migrate great distances to give rise to diverse structures, such as the peripheral nervous system, melanocytes and craniofacial skeleton.

The mechanisms that regulate neural crest EMT remain incompletely understood. Tetraspanin 18 (Tspan18) is a member of the tetraspanin family of transmembrane proteins that have been implicated in cell signaling, motility and adhesion. Tspan18 is abundantly and specifically expressed in chick premigratory neural crest, but is down-regulated when neural crest cells begin to migrate, suggesting that Tspan18 may negatively regulate neural crest migration. Interestingly, mRNA and protein levels of the known neural crest EMT regulator Cadherin6B (Cad6B) decrease following Tspan18 knock-down, while neural crest migration is inconsistently enhanced. In contrast, Tspan18 overexpression impedes neural crest migration, which preliminary results suggest is due in part to Cad6B stabilization. Consistent with the fact that the neural crest transcription factor FoxD3 regulates neural crest cell–cell adhesion and promotes migration, FoxD3 represses Tspan18 expression. Taken together, these data suggest that Tspan18, as a readout of FoxD3, plays an important role in restricting neural crest migration by affecting levels of Cad6B. Experiments are in progress to identify proteins that interact with Tspan18 to gain insight into how dynamic interactions at the neural crest cell surface impact migration, and may provide clues to the mechanisms of EMT during metastasis. Supported by NIH F31 GM087951 and a U of MN Grant in Aid.

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Program/Abstract #84

The retinal pigment epithelium as a model tissue to study the effects of Lrp2/megalin on BMP signaling

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Establishing morphogen gradients is an essential part of development and is orchestrated in part by cell surface receptors. The endocytic receptor LRP2/megalin is expressed on polarized epithelial cells, and binds and endocytoses BMP4 in addition to a large cohort of other factors. Mutations in LRP2 have been shown to cause developmental abnormalities that affect brain, eye and kidney development and function. Likewise, BMP4 alterations are associated with similar developmental disease phenotypes, including cyclopia, holoprosencephaly, anophthalmia–microphthalmia, retinal dystrophy and brain anomalies. In the developing and adult telencephalon, absence of LRP2 has been shown to lead to increased levels of BMP4 which suppresses cell proliferation and induces increased apoptosis. Recently, Lrp2 −/− zebrafish have been shown to exhibit enlarged eye globes, myopia, elevated intraocular pressure and retinal ganglion cell pathogenesis. Here, we use in vivo BRE:dmKO2 reporter zebrafish to assay BMP signaling in the eye in response to retinal pigment epithelium-specific expression of eGFP-tagged Bmp4. We take advantage of the localized expression of Lrp2 in the RPE to study both the persistence of eGFP-Bmp4 and the activation of the Smad1/5/8 pathway in the presence and absence of Lrp2. We show that the ventral aspect of the developing RPE, which shows a low basal level of BRE:dmKO2 activity during development, is ideal to quantify BMP4 activity in response to native and ectopic antagonists.

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Program/Abstract #85

Role of aggrecan in growth plate development: Use of genetically altered mouse models

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Several chondrodysplasias exhibiting altered growth plate (GP) development have been identified in birds and mammals arising from mutations affecting the biochemical properties of aggrecan. One of these, the cmdBC mouse phenotype, results from a complete loss of exons 2 to 18 and the consequent complete absence of mature aggrecan (AGC). In this study, the cmdBC mouse, and a novel AGC mouse model were utilized to dissect the role of ACG in modulating specific signaling pathways during GP development. First, the cmdBC mouse was crossed with the Ptc-LacZ reporter mouse to analyze the range of hedgehog (Hh) signaling. The extent of Ptc expression was greatly affected, with reporter gene expression found centrally located and significantly distant from the perichondrial margin of the GP, indicating a reduced range of Hh signaling. Second, with the goal of elucidating the role of AGC in providing a scaffold for growth factors, the full-length chick Agc gene was cloned and transgenic mice (tg) were generated driving the expression of AGC core protein under the control of the cartilage-specific Col2a promoter. The chick-AGC-specific S103L Mab revealed robust expression of the transgene product in the ECM of the developing GP of the tg mice. Third, attempts to rescue the cmdBC phenotype with the chick-AGC tg lines resulted in a successful mild-to-moderate correction of the cmd skeletal defects; i.e. longer limbs, tail, and snout compared to homozygous cmdBC mice. These mouse models have already proved extremely valuable in understanding the role of AGC in regulating certain signaling pathways during GP development.

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